STATUS OF EXOBIOLOGY PROGRAM

Some elements of the strategy of exobiological investigation have been discussed in previous reports (cf. Westex material as summarized by Space Science Board; Lederberg, J., 1960 "Exobiology - Experimental Approaches to Life Beyond the Earth," Science 132: 393-400, August 12). The first investigations of a new planetary habitat should certainly include a general survey at various magnifications for visual evidence of life; until the results of these are in, it is hard to judge what further steps should be taken in investigating the biology of possible larger organisms. Needless to say, if the first look does give evidence of intelligent culture or of larger organisms, considerable effort would be devoted to characterizing these in later experiments. Meanwhile, a great deal can be planned for and done in microbiological investigation starting from first principles. There are many reasons to justify a strong emphasis on microbiology in planetary investigations just as there are for laboratory studies here. These include:

1) The greater likelihood of a successful result. In some habitats only micro-organisms might be present; on the other hand, it is difficult to conceive of a habitat which contained larger forms but did not contain micro-organisms.

2) Ubiquity. Even on a richly populated planet like ours, micro-organisms can be found in almost any small sample of the atmosphere, the surface dust, or bodies of water. This is much less true of larger organisms.

3) Metabolic diversity. Micro-organisms should afford a wider variety of metabolic capabilities from which we might draw inferences about the comparative biochemistry of the planet.

4) Ease of handling. With any luck at all, at least some species of micro-organisms would be very much easier to cultivate and to contain for detailed experimental investigation.

5) Importance for terrestrial ecology. Both small and large organisms of external origin constitute potential hazards if inadvertently brought to the earth; one might anticipate much greater difficulties in removing micro-organisms and in controlling them if they should escape. Therefore, we should obtain as much information as possible about them in their natural habitat before programming experiments that involve the actual return of planetary samples to the earth.

How then might we procede to investigate the distribution of micro-organisms? We should be prepared to meet the expectations that:

1) A scarcity of moisture may result in a very sparse level of life.

2) The nutrition and biochemistry of the planetary organisms may differ quite markedly from terrestrial ones.

We must, therefore, stress the most efficient methods of detecting micro-organisms and also those methods which do not require a too specific identification of earthly biochemical components. However, it is impossible to do a general experiment in the abstract; and concrete experiments necessarily involve some measured compromises on these principles. Further considerations in experimental design are the necessary limitations on the size of the pay load, the

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necessity for its automatic functioning, and the narrowness of the effective communication channel for returning the data to the earth. We should also keep in mind that apart from the existence of life on another planet, we are most deeply interested in a comparative study of its basic biochemical systems, and in particular, whether other types of compounds can take the place of nucleic acids and proteins of terrestrial organisms.

Proposed methods for exobiological study involve a combination of techniques and a systematic presentation is likely to be rather artificial. The following approaches may be kept in mind.

1) Chemical composition and metabolic effect of the micro-organisms. This approach should be assiduously studied; the chief discouragement is the possible scarcity of material to analyze. However, in combination with, for example, controlled cultivation of the organisms, this approach will probably give us the most detailed information on the intrinsic biochemistry of the exo-organisms. Studies will be made to determine whether such characteristic substrates as ATP (adenosine-triphosphate) are sufficiently rapidly metabolized by small numbers of organisms to warrant further developments along this line.

2) Cultivation of the organisms on prepared media. This is the habitual technique of the microbiologist and will doubtless have very high priority when we can make firmer decisions on exo-microbial nutrition. Where water is a limiting factor in a planetary biology, this may prove to be the most important nutrient. If the communication channel is so narrow that only a few bits are available, an efficient black box design, incorporating a test of microbial growth, and giving a simple yes/no answer might be desired. However, in view of our uncertainties as to the optimal nutrients, it would be wise to test a variety of alternative media. Such a system might be miniaturized by culturing the organisms on moistened spots on a moving tape rather than in bulk culture. The likelihood that the communication channel, though still restricted, might be more ample suggests a more detailed study of the samples. The two most promising procedures appear to be microscopy and spectrophotometry, or some combination of the two.

3) Direct optical examination of the organisms (microscopy and spectrophotometry). These methods involve the measurement of light intensity as a function of position (i.e., the image of a particle) or of the spectral wave length (i.e., its absorption spectrum). If the technical problems can be surmounted, the combination of form and spectral data would give a most powerful method of biological analysis.

a) Type of microscope. Most living cells, including bacteria, are essentially transparent to the customarily visible wave lengths and must be processed in particular ways in order to achieve useful contrast. The traditional way of doing this in the laboratory is by staining, a procedure which can be used for a considerable degree of chemical specification, but which adds to the mechanical complications. Two principal possibilities are left for the observation of unstained objects: microscopy in the ultra-violet at 2600-2800 Å and phase contrast microscopy. The advantage of ultra-violet microscopy would be the relative chemical selectivity at different wave lengths, for example, the very high absorption by nucleic acids at around 2600 Å. However, to take full advantage of this selectivity, additional information on the absorption at one or more other wave lengths would be desirable. Likewise, with phase contrast microscopy, the

greatest information would be obtained from a system which also discriminated between particles which were opaque vs. transparent in ordinary transmission microscopy. Therefore, the efficient use of either UV or phase contrast microscopy suggests the development of some method of color discrimination. This does not necessitate the transmission of full color data, but rather the interposition of a "filter logic" to pass only those signals that fulfill stated criteria (i.e., transparent in visible and opaque in UV or phase contrast). Such finesse should be more appropriate for electronic than photographic recording of the signals, and our preliminary efforts have been directed to evaluating a UV microscope-vidicon chain. Recent work on image-intensifying UV camera tubes (The Westinghouse Ebicon being constructed for the orbital UV telescope) may be extremely useful; other arguments favor the adoption of flying-spot technique into the spectrophotometric-microscope system.

b) Preliminary data reduction in situ. The limited communication channel suggests the most strenuous efforts to eliminate irrelevancy and unwanted redundancy from the transmissions. These arise from at least two sources within the object being looked at:

1) A probable abundance of biologically uninteresting material, especially mineral particles, among which it may be difficult to discriminate cells and

2) High point-to-point correlations in the object being looked at - for example, under phase contrast, bacteria often resemble homogeneous elipsoids.

The waste resulting from the second factor might be minimized by differential and edge-contrast discrimination techniques; the discrimination of color, and perhaps also of form, might be used to monitor a decision as to which elements were worth transmitting. (Needless to say, at least a few unfiltered pictures would be desirable also.) Waste from the first source could be minimized by methods for the concentration of living material and the rejection of other extraneous particles. The cultivation of the organisms, if this succeeds, would be an admirable way to do this. However, in the expectation of possible failure, thought should be given to other methods for the separation of cells from various kinds of debris.

The problems of data reduction summarized here have some analogy to the problems involved in the effective use of satellite reconnaissance (Samos) (cf., Katz's review in Astronautics, July, 1960.).

4) Collection of samples. So far the least attention has been given this problem, perhaps in the anticipation of productive developments in other fields. For example, the auger and corer that have been proposed for the lunar round-trip should be quite applicable to the present purposes except that the specimens would be processed within the payload rather than being returned. In addition, the choice of procedures for collection would depend on the types of examination that were adopted. At the present time, three alternatives are evident. a) The collection of atmospheric dust for direct microscopic examination on adhesive tapes - the flypaper proposal. b) The collection and concentration of larger amounts of atmospheric dust by impaction or filtration - methods now in standard use in aerial microbiology. c) The soil sampler.

The first method might be applicable for direct inspection and also cultivation on the tapes themselves. However, if the payload weight permits, it would be safer to collect a larger sample and enrich it by the flotation of cells in a dense medium as described further.

5) Present judgments. At the present time, I would suggest a program with the following objectives. a) The collection of from 10-100 grams of dust or soil. b) The fractionation of the sample by flotation and c) direct examination of the organic fraction by 1) phase contrast/transmission dual microscopy and 2) microspectrophotometry between 2400 and 8000 Å, either on single particles or on cleared samples of the organic fraction. c) Data reduction and programming to transmit only the more likely observations. Part of the sample should also be cultivated on a nutrient tape and then also examined in like fashion.

It will be difficult to make more refined choices and decisions until the hardware has been designed and constructed to the point where it can be tried out extensively on a larger variety of terrestrial samples. One of our necessary and most important functions long before space flight will be the calibration of these procedures and the search for the kinds of artifacts that must be avoided.

6) Further refinements and later experiments. With appropriate support and cooperation of industrial organizations a workable system incorporating some or all of the above elements should be ready in ample time for Saturn flights to Mars circa 1965. If time still permits, additional refinements can be added for the first missions and must be planned for succeeding ones. These might include a) More complete cytochemical tests of observed particles, in particular for DNA and for proteins. It should be possible to adjoin these tests to the microscopic observation. b) Command vs. automatic control of the instrumentation to allow for unexpected developments at the target. c) Larger scale enrichments for Martian organisms. These might depend on good guesses or acquired information on the chemistry of the Martian surface and on the physiology of the Martian organisms, and could then expand this knowledge.